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Study of Gibberellic Acid Production by Immobilized Cells of Fusarium Moniliforme

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ABSTRACT

Production of Gibberellic acid using immobilized cells of Fusarium moniliforme Sheldon, isolated from wilted sugarcane plants, by employing various entrapment agents at 2.0 gm % level viz. sodium alginate, K – karrageenan and agar agar was carried out. Among the various gel entrapment agents evaluated, sodium alginate was found to be superior which has given 230 µg/ml GA3 which was 1.5 to 2.0 time higher production than other agents. The GA3 productivity increases on 5th day and 6th day but decreases on 7th day in all the entrapment agents. Sodium alginate was better as it resulted in comparatively firm and stable beads. When the effect of bead size on GA3 production using sodium alginate was investigated it was found that the maximum GA3 production was observed in the beads of 2.5 mm diameter which was 300 µg/ml GA3. Also as the size of beads increases the GA3 production decreases. This may be due to reduction in the effective area for diffusion as the bead diameter increases. The GA3 production was not improved by immobilization. The level of GA3 productivity decreased in compared to the free cells which had given 315 µg/ml GA3. This may be due to the methods of immobilization used, which may have affected the metabolic properties of the cells. When comparison was made with ssf technique, it is clear that the ssf is better method for GA3 production and should be preferred for reducing the cost of GA3 production.

KEYWORDS : Fusarium moniliforme, Gibberellic acid, Submerged Fermentation, Wheat bran extract, Immobilization

INTRODUCTION :

Fusarium moniliforme is reported to produce Gibberellic acids (GAs) which are a group of diterpenoic acids that function as plant growth regulators influencing a range of developmental processes in higher plants including stem elongation, germination, dormancy, flowering, sex expression, enzyme induction and leaf and fruit senescence. The origin of research in to gibberellins can be traced to Japanese plant pathologists who were investigating the causes of the "bakanae" (foolish seedling) disease which seriously lowered the yield of rice crops in Japan, Taiwan and throughout the Asian continent (Kurosawa E. 1926).

The first paper on the cause of bakanae as published in 1898. By Shotaro Hori who demonstrated that the symptoms were induced by infection with a fungus belonging to the genes *Fusarium*, probably *Fusarium heterosporium* Nees. Subsequently, Elichi Kurosawa (1926) found that culture filtrates from dried rice seedlings caused marked elongation in rice and other sub-tropical grasses. He concluded that bakanae fungus secretes a chemical that stimulates shoot elongation, inhibits chlorophyll formation and suppresses root growth.

Teijiro Yabuta initiated work on the isolation of the active component using the fungal strains provided by Kurosawa. As a result, non-crystalline solid was obtained from the culture filtrate that stimulated the growth of rice seedlings. This compound was named gibberellin by Yabuta in 1935; the first use of the term "gibberellin" in the scientific literature.

In 1938, Yabuta and his associate Yusuke Sumiki finally succeeded in crystallizing a pale yellow solid to yield gibberellin A ($C_{22}H_{26}O_7$) and gibberellin B ($C_{19}H_{22}O_3$). The names were subsequently inter changed in 1941 and the original gibberellin A was found to be inactive.

In the United States, the first research on gibberellins began after the Second World War by a research unit at Camp Dietrick, Maryland. In 1950, John E. Mitchell reported optimal fermentation procedures for the fungus, as well as the effects of fungul extracts on the growth of bean (Viciafaba) seedlings (Mitchell & Angel 1951). At about the same time in the UK, a team of researchers at Akers Research Laboratories (ICI) isolated a new gibberellin which was given the name "Gibberellic acid". This compound had physical properties different from the Japanese gibberellin A (Curtis & Cross, 1954). Samples were exchanged between Stodola and Grove and "Gibberellic acid" and gibberellin–X were found to have identical chemical and physical properties and the name Gibberellic acid was accepted by both groups. A structure for Gibberellic acid was proposed in 1956 but later revised (Grove–1961).

Fermentation mechanism of GA production is very complex as it is a secondary metabolite.

Kumar and Lonsane (1988) studied gibberellic acid production using immobilized growing cells of *Gibberella fujikuroi* in batch and semi continuous cultures. The Gibberellic acid production was affected by the immobilization agent used, nature and age of cells, mycelial density, size of beads and inclusion of linseed oil. The beads prepared could be recycled without affecting productivity in semi continuous culture. The rate of production of gibberellic acid was 0.58 – 0.66 mg/liter/hr. An inverted conical fluidized bioreactor, based on the design employed in continuous plant cell culture, was adopted.

Nava saucedo *et. al.* (1989) carried out experiments on continuous production of gibberellic acid in a fixed bed reactor by immobilized mycelia of *Gibberella fujikuroi* in calcium alginate beads. The production of Gibberellic acid by immobilized mycelia was maintained over a hundred days in a tubular fixed-bed reactor. Gibberellic acid production in both free and immobilized mycelium shake flask cultures was 0.384 and 0.408 mg GA_3 /gm biomass /day, respectively. Whereas in the continuous system the Gibberellic acid production was about twice as large for a similar biomass i.e., 0.768 mg GA_3 / gm biomass / day. Several factors affecting the overall productivity of the immobilized systems were found to be the quality and the quantity of mycelia in the biocatalyst beads and the immobilization conditions.

Studying production of gibberellic acid in *Gibberella fujikuroi* adhere on to polymeric fibrous carriers, Lu Z. X.,Xie Z. C. and Kumakura M. (1995) concluded that GA production in cells immobilized with the carrier–covered copolymer 0f hydrophilic hydroxyl ethyl acrylate and hydrophobic trimethyl propane triacrylate was higher than that in the free cells. The immobilized cells produced GA with higher stability. Gibberellic acid productivity by immobilized cells varied with the chemical nature of the carriers covered by the polymers.

Recently, the immobilization of whole growing cells has been studied, mainly because of its economic potential. Advantages of an immobilized system based on living cells include their active metabolic ability to synthesize various useful and complicated bio products using the multi-enzyme steps, and the regeneration capability to prolong their catalytic life (Vignoli et al., 2006). Immobilization of microorganisms with a number of techniques including encapsulation, entrapment in polymer gels, and adhesion onto the surface of carriers has been reported (Aykut et al., 1988; Mozes and Rouxhet, 1984). The viability of using loofa sponge as a carrier for microbial cells was studied successfully (Vignoli et al., 2006; Saudagar et al., 2008). Production of GA from milk permeate by a selected mutant of *F. moniliforme* immobilized on loofa sponge discs to ascertain semi-continuous method was

successfully done (Meleigy and Khalaf, 2009).

MATERIALS AND METHODS:

1: PRODUCTION OF GA_3 BY IMMOBILIZED CELLS OF *FUSARIUM MONILIFORME* USING DIFFERENT ENTRAPMENT AGENTS:

The method described by Kumar and Lonsane (1988) was used for this study

1.1: MYCELIAL CELL PRODUCTION:

Fusarium moniliforme was grown in Czapek Dox liquid medium in shake flasks at 28 ± 1 °C till the initiation of GA_3 production phase, i.e. 3 days. The mycelial cells were separated by centrifugation at 5000 rpm for 20 minutes and washed twice with physiological saline of pH – 6.0 and used in the immobilization experiments. All the operations including the immobilization and storage of beads were done under aseptic conditions.

1.2: TECHNIQUE OF IMMOBILIZATION:

USING SODIUM ALGINATE:

1.0 gm wet mycelial cells were suspended uniformly in 10 ml 2.0 % w/v solution of sodium alginate at 28 °C . The mixture was added drop wise, using a syringe, to 2.0 % w/v solution of $CaCl_2$ under gentle stirring at 28 °C to obtain spherical beads.

USING K – KARRAGEENAN:

1.0 gm wet mycelial cells were suspended uniformly in 10 ml 2.0 % w/v K – karrageenan at 45 °C . The mixture was added drop wise, using a syringe, to 2.0 % w/v solution of KCl.

USING AGAR AGAR:

1.0 gm wet mycelial cells were suspended uniformly in 10 ml 2.0 % w/v agar agar at 43 °C . The mixture was added drop wise, using a syringe, to cold water under gentle stirring to obtain spherical beads. After 1 hour the beads were removed, washed twice with sterile distilled water and used.

1.3: BATCH CULTURE:

The immobilized growing cells, prepared as described above were used for the production of GA_3 in 40 times diluted wheat bran (WB) extract medium containing 2.0 % sucrose with pH – 5.5.

Immobilized cells were added to 100 ml of above mentioned medium in 500 ml Erlenmeyer flasks and incubated at 28 ± 1 °C on rotary shaker at 150 rpm speed. The amount of GA_3 produced was estimated at 120 , 144 and 168 hours.

1.4: EXTRACTION OF GA_3 FROM FERMENTED BROTH:

The fermented broth was filtered through Whatman no – 1 filter paper and GA_3 from the filtrate was extracted at pH -2.5 using 2.0 N HCl by adding ethyl acetate in three stages to obtain 45 ml extract from 50 ml filtered broth. The extract was evaporated to about 5.0 ml .GA_3 was estimated by colorimetric technique and mycellial dry weight was determined.

1.5: ESTIMATION OF GA_3 BY SPECTROPHOTOMETRIC TECHNIQUE:

The concentration of GA_3 in ethyl extract was estimated using spectrophotometric method described by Berriso *et. al.* (2004) at 254 nm in a UV/Visible spectrophotometer (Elico make).

1.6 : EFFECT OF BEAD SIZE ON PRODUCTION OF GA_3 BY IMMOBILIZED MYCELIA:

The mycelia of *Fusarium moniliforme* were produced and immobilized using sodium alginate. To vary the size of beads, syringe with three different sized needles were used to prepare the beads. All the three different size beads were taken in separate sets of batch culture flasks and GA_3 production was checked.

RESULTS AND DISCUSSIONS:

2.1: GA3 PRODUCTION BY IMMOBILIZED CELLS OF *F. MONILIFORME*:

Production of GA3 using immobilized cells of *F. moniliforme,* Sheldon by employing various entrapment agents was carried out and the results are presented in figure 1.

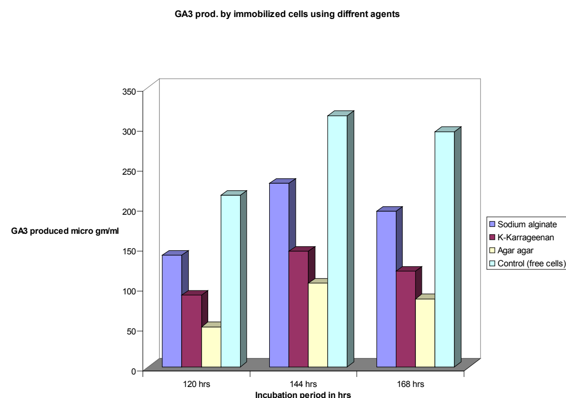
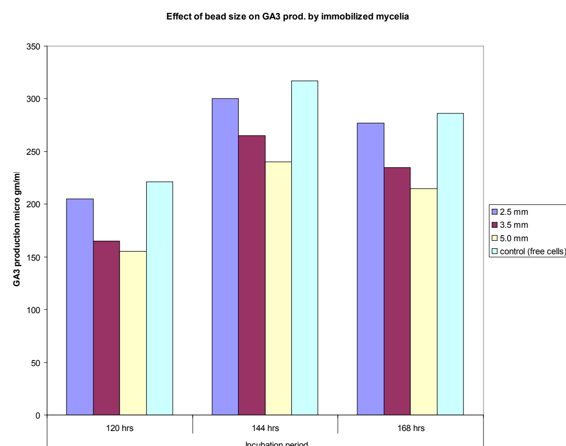


Figure – 1- GA3 production by immobilized cells using different entrapment agents.

Among the various gel entrapment agents evaluated sodium alginate was found to be superior. which has given 230 µg/ml GA_3 which was 1.5 to 2.0 times higher production than other agents. The GA_3 productivity increases on 5th day and the 6th day but decreases on 7th day in all the entrapment agents. Sodium alginate was better as it resulted in comparatively firm and stable beads. Similar results were obtained by Kumar and Lonsane (1988) where they could produce gibberellic acid at 0.58 – 0.66 mg/liter/hr rate. Nava saucedo *et. al.* (1989) carried out experiments on continuous production of gibberellic acid in a fixed bed reactor by immobilized mycelia of *Gibberella fujikuroi* in calcium alginate beads. The production of Gibberellic acid by immobilized mycelia was maintained over a hundred days in a tubular fixed-bed reactor. Gibberellic acid production in both free and immobilized mycelium shake flask cultures was 0.384 and 0.408 mg GA_3 / gm biomass /day, respectively. Whereas in the continuous system the Gibberellic acid production was about twice as large for a similar biomass i.e., 0.768 mg GA_3 / gm biomass / day. In their experiment Samir A. Meleigy and Mahmoud A. Khalaf (2009) got quite contrasting results and they concluded that GA production from milk permeate with immobilized mycelia of mutant c-14 on loofa sponge could reach 2.4 gl/1, more than 2.8-times greater than previously reported by free cells. Doaa A.E.M.Sleem (2013) in his experiment produced 2.51 g/l GA_3 compared to 2.30 g/l by free cells of *F.moniliforme*.

Figure – 2 Effect of bead size on GA3 production by immobilized cells of *F.moniliforme.*



When the effect of bead size on GA_3 production using sodium alginate was investigated it was found that the maximum GA_3 production was observed in the beads of 2.5 mm diameter, figure 2. Also as the size of beads increases the GA_3 production decreases. This may be due to reduction in the effective area for diffusion as the bead diameter increases. Similar results were obtained by Kumar and Lonsane (1988) using immobilized growing cells of *G. fujikuroi* P – 3 for GA_3 production. Nava saucedo *et. al.* (1989) concluded that several factors affecting the overall productivity of the immobilized systems were found

to be the quality and the quantity of mycelia in the biocatalyst beads and the immobilization conditions.

The GA_3 production was not improved by immobilization. The level of GA_3 productivity decreased in compared to the free cells which produced 315 µg/ml. This may be due to the methods of immobilization used, which may have affected the metabolic properties of the cells. When comparison was made with ssf technique, it is clear that the ssf is better method for GA_3 production and should be preferred for reducing the cost of GA_3 production.

SUMMARY:

Gibberellic acid production was tested through immobilization of cells using gel entrapment agents like Agar agar, Sodium alginate and K-Karrageenan. Sodium alginate entrapment gave higher production of gibberellic acid 300 µg/ml GA_3 which was little less than the production in control 317 µg/ml GA_3. When the effect of bead size on gibberellic acid production was studied, smaller beads gave better production. The information obtained through detailed studies of GA3 production in smf, and through immobilization can be exploited at the commercial level as GA_3 is a plant growth hormone.

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